Summary of Comments on the NTP Research Concept: Diethyl Phthalate (DEP)

Introduction

Diethyl phthalate (DEP) is used primarily as a solvent in a number of applications, including personal care products and fragrances. Members of the phthalate class have been found to produce a range of effects on reproductive development in rats due to their ability to interfere primarily with androgen action. Human exposures to DEP are higher than for other phthalates. The research concept proposed conducting a robust multigenerational study in the rat incorporating end points known to be sensitive to antiandrogenic effects of phthalates.

Importance to Human Health

The concept document verifies the importance of diethyl phthalate due to ubiquitous human DEP exposure. Its marker metabolite, monoethyl phthalate (mEP), has the highest human urinary concentrations compared to all other phthalates measured by the CDC. In addition, human epidemiologic studies have shown associations between mEP and SHBG and altered free testosterone: LH.

NTP has previously identified significant reproductive changes, including lower sperm counts, in a continuous breeding study in mice. Although adverse effects were only noted at the highest dose, mice are noted to be resistant to reproductive toxicity from phthalates. The notation that the effect on the F1 generation was greater than on the F0 generation is important because phthalates in general have had increased effects in subsequent generations and past negative study designs have not been adequate to address this issue.

Study Design

Several studies have been conducted with DEP and have shown no effects indicative of antiandrogenic activity, including a multigeneration study (Fujii et al. 2005), a perinatal study (Gray et al 2000) and a pubertal study (Foster et al. 1980). Although multiple studies in rats have been performed, as the concept document correctly states, several significant design flaws are noted. These include: inadequate power to test a null hypothesis with a very limited sample size (In some cases, only 1 male and 1 female pup. In others, only 3 litters were evaluated). Because the purpose of the NTP studies is to address flawed designs in previous negative studies, it would be helpful if the concept document included sufficient detail to address the prior flaws. Thus, the comments below include more detailed comments.

How will the dosing range be determined and will it be likely to generate mEP concentrations similar to those seen in relevant subsets of humans? Pregnant women appear to have the highest urinary mEP concentrations with 95%ile and maximum values of 5.520 and 30.5 mg/mg creatinine reported by Adibi et al. (2003), and Marsee et al. (2006), respectively. The corresponding 95%ile and maximum human exposure estimates were 0.183 and 1.263 mg/kg/d, respectively. Unfortunately, no studies have been published of mEP concentrations in preterm infants, a group that also may have greater developmental susceptibility. For other phthalates, preterm infant's measured urinary mEP concentrations were three orders of magnitude higher than that published for older children.

It is clearly stated that a significant number of animals will be evaluated not only at weaning, but also at adulthood. The list of endpoints is not included in the concept document. Because missing endpoints in prior negative studies is part of the rationale for the proposed work, these merit clear discussion even though they are well recognized by NTP leadership as published in Foster (2006). Structural endpoints to be considered for inclusion are permanent nipples, areolas in infants, anogenital distance, hypospadius and epispadias, agenesis of sex accessory glands, testis or epididymal sperm counts, histological analysis of testes and epididymis, prostatitis, and the weight of androgen sensitive organs (levator ani), free testosterone, LH, SHBG, insulin like factor 3 and perhaps inhibin B. Alterations in free testosterone: LH and SHBG have been associated with increased mEP in humans and, thus, these endpoints would be valuable mechanistically.

Will gene expression changes be evaluated in these studies or would such studies be considered as second tier? As a single rat study using a relatively high DEP dose did not show changes in gene expression (Liu et al, 2005), such studies could be considered lower priority, but perhaps tissues should be saved if needed for analysis.

In addition, the NTP proposes toxicokinetic studies (including sampling of amniotic fluid) to allow calculations of fetal exposure and comparison to human data. These are essential for validating the relevance of the animal model. Will there be an arm that includes both in utero and early postnatal exposure?

One possible explanation for putatively positive findings in humans and unimpressive results in rodents is that there is a high prevalence of zinc deficiency in humans and some literature that DEP may work by a zinc dependent process. Thus, a rodent model with a zinc deficiency diet arm is needed.

The design of the toxicokinetic assessment, that is briefly mentioned, is a crucial component of this project, as it will allow comparison between rat and reported human levels. This study will also add to our knowledge base of the structure-activity relationship to aid in predicting the antiandrogenic activity of various phthalates and prioritizing future research needs in this area.

There is not a sufficiently strong case made in the proposal for the need for a multigeneration reproduction study to fill this data gap. The major concern from exposure to phthalates is interference with androgen action during male reproductive development. This question has been investigated successfully for other phthalates using a shorter (in utero or perinatal exposure), less complex study design, which could also be applied to studying the effects of DEP, without having to conduct a multigeneration study. Consideration should therefore be given to a shorter, less complex study design than the multigeneration reproduction study. An example of an alternative design would be to dose in utero only (from implantation to just prior to parturition or up to weaning) and evaluate more offspring (more than the standard one per litter) postnatally through adulthood (developmental landmarks, sexual maturation, pathology, possibly early postnatal testosterone levels?). Consideration should be given to including other phthalates that are suspected of being inactive (e.g. DMP) or weakly active (e.g. DINP) or including a positive control group (e.g. DEHP, DBP). The feasibility of including additional groups/compounds is greater if the alternative design is adopted instead of the multigeneration design.

A recent epidemiological study (Swan et al. 2005) found a positive correlation of reduced anogenital index (anogenital distance/bodyweight) in infant boys with increasing phthalate metabolite urinary levels in mothers. This study has raised concern about potential effects upon development of current human exposures to phthalates. Given humans are exposed to many phthalates and that multigenerational studies in animals exposed to DEP at up to 20,000 times the exposure level of humans have not found comparable effects in animals, it seems unlikely that the proposed research will contribute much to resolving the concern raised by the Swan et al. findings. In contradistinction to what was stated in the concept paper, it does not appear that this response has been confirmed in other studies. Moreover, the Swan study only reported results for anogenital distance normalized by body weight, which appears to be a non-standard method of normalization.

It is suggested that a robust epidemiological study to verify the findings in the Swan et al. study would be more useful in addressing the concern for effects in humans that the proposed mutigenerational animal studies. Such a study could correlate non-invasive indicators of developmental effects in infants, such as anogenital distance, and using multiple methods for normalizing responses, with measures of urinary and/or serum phthalate metabolites in mothers. If such effects are found additional followup of the infants could be conducted to determine whether the observed effects result in adverse effects later in life.

Potential Significance of Proposed Research

The rationale for the proposed research captures both the data gaps in hazard evaluation as well as the concern that stems from the potential significant human exposure to DEP. Recent data suggests that human exposure to DEP may be higher than for other phthalates, and this is a concern, given the limited information available for the antiandrogenic potential of DEP. This research program is very timely, as it will provide toxicology data to put into perspective recent data about a possible correlation between high human exposure to DEP and androgen status in boys.

In general, the NTP proposal of a multi-generational study using a wide dosing range, with full assessment of presumably the F1 and F2 generations is appropriate in scope and is required to sufficiently address the current gap in knowledge. Additional hazard evaluation is needed because the earlier perinatal study had limited statistical power (only 3 litters evaluated), the pubertal study did not cover the critical window (in utero) and there is concern that the multigenerational design did not evaluate sufficient pups per litter. Taken together these considerations make a good case for the need for more toxicological research on DEP.

However, in the multigeneration study by Fujii et al. there was no detectable effect on male reproductive development at a very high DEP intake (~1 g/kg/day), which should lower the concern about the sensitivity of the study. If such effects were detected in an alternative design where a greater number of pups are examined, the NOAEL is still likely to be very high compared to human exposure levels. Thus, given that a number of multigenerational studies have already been conducted in both mice and rats, and at extremely high doses compared to human exposures, there are doubts that the proposed study, whatever the outcome, would do much to advance our understanding of the ability of diethyl phthalate or related compounds to cause developmental effects in humans at current exposure levels. Thus it seems that a robust epidemiological study is likely to be much more effective in resolving the concerns for health effects in humans from phthalate exposures than the proposed multigenerational study in rats. An additional limitation of the proposed rat study for resolving concerns in humans is that it only involves a single phthalate, albeit the one to which humans are most highly exposed.

References

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